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Pseudomonas Treatment Composition and Method

This application claims priority to U.S. Provisional Application Serial No. 60/089,155 filed June 12, 1998, which is hereby incorporated by reference.

Field of the Invention

The present invention relates to a novel composition and method for treatment and prevention of infection by *Pseudomonas aeruginosa*.

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Background of the Invention

Pseudomonas aeruginosa is a significant opportunistic pathogen that causes a variety of life-threatening infections in immunosuppressed or immunocompromised patients [1-4]. Individuals who are at risk of developing *P. aeruginosa* infections include cystic fibrosis patients, burn patients, severe neutropenic patients (e.g., cancer patients receiving chemotherapy) and intensive care unit patients receiving respiratory support. The cost of these infections is high, >60,000 lives per year in North America and about $5 billion/year in health care costs.

The first step in the *Pseudomonas* infection process appears to be the attachment to the host cell. This attachment is mediated by pili on the surface of the bacterium [2, 5, 6]. *P. aeruginosa* uses several adhesins to mediate attachment to mucosal surfaces, but analysis of the binding properties of the adhesins [1, 7, 8] and binding competition studies [9] indicate that the pilus is the dominant adhesin responsible for initiating infections [1].

P. aeruginosa pili have a structure resembling a hollow tube of about 5.2 nm in outer diameter, 1.2 nm in central channel diameter, and an average length of 2.5 μm [10-12]. The pilus of *P. aeruginosa* is composed of multiple copies of a 13-17 kDa monomeric protein subunit called pilin, which are capable of self-assembling into pili.

The C-terminal region of the pilin monomer contains the epithelial cell binding domain [5, 12], and is semiconserved in seven different strains of this bacterium [13, 14]. This semiconserved region has also been shown to bind to a minimal structural carbohydrate receptor sequence, β-GalNAc(1-4)βGal, found in glycosphingolipids, specifically asialo-GM1 and asialo-GM2 [15, 16]. There is evidence that pili binding to a host cell is mediated multivalent binding of C-terminal binding domains in each pili to epithelial-cell receptors, with such binding serving to mobilize receptors on the cells. This, in turn, may be responsible to cytokine, e.g., IL-8 production by the host cells and consequent inflammatory response.

The C-terminal disulfide-bridged 17-residue region of the PAK pilin is known to be important in raising antibodies that block binding of both bacteria or their pili to epithelial cells [6, 17, 18]. Both monoclonal antisera generated from *P. aeruginosa* pili or polyclonal antisera generated from synthetic peptides representing the receptor binding domain of the pathogen have been shown to be efficacious in preventing infection [19].

The ability of antibodies produced against the C-terminal pilin-peptide domain to effectively inhibit *Pseudomonas* infection has been demonstrated (see, for example, U.S. Patent No. 5,468,484), and the use of the pilin-peptide domain for use in vaccination against *Pseudomonas* infection has also been demonstrated, e.g., U.S. Patent Nos. 5,445,818, 5,494,672, and 5,612,036.

It would also be desirable to directly treat an existing *Pseudomonas* infection, or to treat an individual at risk of *Pseudomonas* infection prophylactically. Although intact pili have been proposed

for this purpose, this method is limited by the fact that isolated, self-assembled pili have the ability to provoke a strong inflammatory response. Alternatively, the C-terminal pilin peptide has been proposed for this purpose, but this approach is limited by the relatively weak binding of the peptide to the host-cell receptor sites.

Summary of the Invention

The invention includes, in one aspect, a composition for use in treating or preventing infection by *Pseudomonas aeruginosa*. The composition comprises a *P. aeruginosa* pilin protein having an N-terminal peptide region modified to prevent self assembly of the peptide. The peptide may be formulated in a pharmaceutically acceptable carrier, such as an aerosolizable liquid or particle vehicle, or an injectable solution.

In one general embodiment, the modified N-terminal peptide region lacks an N-terminal portion of native *P. aeruginosa*, preferably the first 15 up to the first 40 amino acids residues of native *P. aeruginosa*, more preferably the first 25 up to the first 30 amino acids.

In another general embodiment, the N-terminal region is modified, e.g., by amino acid substitutions, to prevent or inhibit alpha-helix formation in the N-terminal region, thereby preventing the pilin peptide from self-assembling.

In still another embodiment, the N-terminal region of the pilin peptide is replaced by a peptide moiety capable of forming a coiled-coil heterodimer or homodimer structure with an oppositely charged or identical alpha-helix forming peptide moiety, as represented by a so-called leucine zipper peptide. The modified pilin peptide can form dimeric structures which have higher binding affinity to host cells than the corresponding monomer, by virtue of divalent binding, but which are less inflammatory than intact pili, due to the reduced degree of mobilization of host-cell receptor sites, relative to that produced by binding of intact pili. Further, the dimeric construction allows pilin peptides from two different *Pseudomonas* strains to be assembled in dimeric form, or a combination of a single pilin peptide and another therapeutic agent, e.g., an antibacterial agent carried in cleavable form on a carrier peptide which forms the other monomer in the dimeric structure.

The modified pilin peptide may be further modified, in accordance with the invention, to reduce or eliminate immunogenicity in the target organism, e.g., humans.

In accordance with another aspect of the invention, the composition is used in treating or preventing *P. aeruginosa* infection in a subject a pharmaceutically effective amount of the modified *P. aeruginosa* pilin protein to the subject. The peptide is administered, for example, by formulating the peptide as a liquid or particulate aerosol, and delivering the aerosol to the subject's airway., or by intravenous administration.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction with the accompanying drawings.

Brief Description of the Drawings

Figs. 1A-1E give the nucleotide and corresponding amino acid sequence of an exemplary truncated pilin protein from K122 (1A), PAK (1B), PAO (1C), P1 (1D, and KB7 (1E), where the nucleotide and polypeptide sequences from K122, PAK, PAO, P1, and KB7 are identified by SEQ ID NOS: 1 and 2, SEQ ID NOS: 3 and 4, SEQ ID NOS: 5 and 6, SEQ ID NOS: 7 and 8, and SEQ ID NOS: 9 and 10, respectively;

Figs. 2A and 2B show vector constructs designated pDLB42 and pDLB43 containing an N-terminal H coil-truncated PAK fusion peptide (2A), and an N-terminal E coil-truncated PAK fusion peptide (2B);

Figs. 3A and 3B show the nucleotide and polypeptide sequences of an exemplary N-terminal H coil-truncated PAK fusion peptide (3A), and an exemplary N-terminal E coil-truncated PAK fusion peptide (3B) in the vector constructs in Figs. 2A and 2B, respectively, where the nucleotide and polypeptides sequences are identified by SEQ ID NOS: 11 and 12, and SEQ ID NOS: 13 and 14, respectively;

Figs. 4A and 4B show nucleotide and polypeptide sequences of an exemplary N-terminal H coil-truncated K122 fusion peptide (4A), and an exemplary N-terminal E coil-truncated K122 fusion peptide, respectively, where the nucleotide and polypeptides sequences are identified by SEQ ID NOS: 15 and 16, and SEQ ID NOS: 17 and 18, respectively;

Figs. 5A and 5B show nucleotide and polypeptide sequences of an exemplary N-terminal H coil-truncated PAO peptide (5A), and an exemplary N-terminal E coil-truncated fusion PAO peptide, respectively, where the nucleotide and polypeptides sequences are identified by SEQ ID NOS: 19 and 20, and SEQ ID NOS: 21 and 22, respectively;

Fig. 6 illustrates steps in the purification of the K122 truncated pilin protein from Fig. 1A;

Fig. 7 is a plot of size exclusion chromatography of the truncated K122 pilin protein from Fig. 1A;

Fig. 8 is a plot of the competitive inhibition of biotinylated PAK pili binding to immobilized asialo-GM_1 by the truncated K122 pilin protein of Fig. 1A; and

Fig. 9 illustrates the ability of a modified pilin protein formed in accordance with the invention to prolong survival in a mouse model of *Pseudomonas* infection;

Detailed Description of the Invention

The first section describes exemplary modified *Pseudomonas* pilin peptides formed in accordance with the invention, and expression vectors for recombinant expression of the proteins. The second section provides procedures for constructing a model expression vector for a truncated PAK pilin protein, for isolating the protein, and a competitive binding assay for characterizing the modified protein's ability to bind to ASIALO-GM_1 glycosphingolipid. The method of treating or preventing *Pseudomonas* infection, in accordance with another aspect of the invention, and the effect of the treatment in a model animal system, is given in the third section.

I. Modified Pilin-Peptide Compositions

The invention takes advantage of the observation herein that the *P. aeruginosa* pilin protein can be modified to prevent self-assembly, i.e., oligomerization, by modifying the N terminus of the protein to prevent alpha helix formation, and the further observation herein that a monomeric or dimeric form of the pilin peptide is effective in treating or as a prophylactic for *Pseudomonas* infection, but without, or with a significantly reduced inflammatory response relative to intact pili. The N-terminal peptide modifications contemplated herein are of three general types:

(i) amino acid changes which alter the peptide's ability to form α-helical structures in the N-terminal portion of the protein, preferably in the first 20-40 residues of the e.g., and (ii) deletions in the N-terminal portion of the peptide, e.g., a deletion of the first 15 to the first 40 N-terminal amino acids, preferably the first 25 to first 30 N-terminal amino acids; and

(iii) replacement of the N-terminal portion with an alpha-helical coiled-coil homodimer or heterodimer sequence.

Amino acid modifications, e.g., substitutions, deletions, or additions in the N-terminal portion of the peptide effective to produce a non-self-assembling peptide can be determined from known physical interactions that determine the properties of proteins, and from the conformational properties of polypeptide chains. In particular, modifications that affect the ability of the N-terminal portion to disrupt α-helix formation in the first N-terminal 30 amino acid region of the protein will generally be pertinent. Introduction of Pro residues, in particular, in this segment of the protein will significantly disrupt α-helix formation, but other residues that tend to destabilize α-helices, e.g., groups of Gly, His or Asn, are also contemplated (see, for example, the discussion in Proteins, supra, pages, 182-186, and refs. 35 and 36). For example, a string of continuous Gly, His, or Asn residues, e.g., 3-5 residue string, will effectively prevent alpha helix formation, as will periodic Pro residues, e.g., every 5-7 residues.

The amino acid sequences of several *Pseudomonas* pilin peptides have been reported [e.g., 37-41]. Further, there is a large body of literature references that provide guidance as to the types

and frequency of residues that will effectively prevent alpha-helix formation are available. From these references, one may construct specific amino acid substitutions, deletions, or additions that would be predicted to eliminate or reduce the tendency of alpha-helix formation in the first 15-40 residues of a selected pilin peptide. Alternatively, a variety of computer algorithms designed to predict secondary structure may be employed to determine whether given amino acid substitutions in the N-terminal region of a selected *Pseudomonas* pilin peptide are likely to be effective in blocking alpha-helix formation [e.g., 25-32].

Alternatively, the N-terminal portion of the peptide may be deleted, to produce a pilin protein whose N-terminal region is lacking a critical α-helical forming portion. As noted above, the deletions in the N-terminal portion of the peptide, are preferably the first 15 to the first 40 N-terminal amino acids, preferably the first 25 to first 30 N-terminal amino acids. In one exemplary peptide described below, the pilin protein from strain K122 has N-terminal residues 1-28 deleted. This peptide is identified below as K-122-4. The polynucleotide and corresponding polypeptide sequences of the modified protein are given in Fig. 1A, and are identified herein as SEQ ID NO:1 (polynucleotide sequence) and SEQ ID NO: 2 (polypeptide sequence). The first five amino acid residues in the polypeptide sequence are not native to the K122 sequence, but are derived from an intrinsic coding sequence of the expression vector. The C-terminal residue of the polypeptide is the Pro residue immediately upstream of the two stop OCH codons; that is, the polypeptide sequence identified by SEQ ID NO:2 does not include the residues Ser-Ser-Lys-Leu-Gly downstream of the stop codons.

Similar polynucleotide and polypeptide sequence for truncated pilin peptides from PAK, PAO, P1, and KB7 *Pseudomonas* strains are given in Figs. 1B-1E, respectively. The polynucleotide and polypeptide sequences for the truncated pilin peptide from strain PAK are identified as SEQ ID NOS:3 and 4, respectively (Fig. 1B; for the truncated pilin peptide from strain PAO, SEQ ID NOS:5 and 6, respectively (Fig. 1C); for the truncated pilin peptide from strain P1, SEQ ID NOS:7 and 8, respectively (Fig. 1D); and for the truncated pilin peptide from strain KB7, SEQ ID NOS:9 and 10, respectively (Fig. 1E).

The example below illustrates the recombinant production of the above truncated pilin protein, designated K122-4, truncated to delete its N-terminal 28 amino acid residues. It will be recognized by one skilled in the art that a variety of procedures are available for producing *P. aeruginosa* pilin protein with a modified N-terminal region. For example, references 20-26 disclose various *P. aeruginosa* pilin protein genes. Reference 27 details methods for expressing pilin peptide in *E. coli*. These references are incorporated herein by reference.

It will be further appreciated that methods for modifying the N-terminal region of a pili protein gene, to achieve a desired modification in the protein, are well within the skill of persons skilled in the art. For example, site directed mutagenesis, including substitution, deletion, and

addition mutations of the gene sequence can be carried out by well known methods, e.g., involving PCR primers. Similarly genes with various-length truncations can be prepared by standard means, as exemplified below.

Figs. 1A-1E illustrate exemplary coding sequences for N-termina! region truncated pilin peptide. For compositions in which one or amino acids are substituted in the first 20-40 residues, to prevent alpha-helix formation in the N-terminal region, the peptide has known pilin-peptide sequences (see references above relating to *P. aeruginosa* pilin sequence, e.g., references 5, 12, 13, and 14), but modified to contain amino acid substitutions or additions, e.g., Pro residues or Gly strings at suitable residue positions. For producing such modified proteins recombinantly, one can suitably modify the coding sequence for the corresponding pilin peptide, using standard techniques, such as site-directed mutagenesis, PCR amplification with suitable-sequence primers, or solid-phase synthesis.

In the third general embodiment of the composition of the invention, the N-terminal region of a pilin peptide, e.g., the first 15-40 residues, is replaced by a peptide segment capable of forming a coiled-coil homodimer with an identical peptide segment, or a heterodimer with an oppositely charged peptide segment. Peptides with this coiled-coil dimer forming property have been disclosed, e.g., in PCT applications WO 97/12988 and WO 95/31480, which are incorporated herein by reference.

Exemplary coiled-coil peptides are referred to herein E coils, referring to negatively charged subunits whose charge is provided predominantly by glutamic acid residues, and K coils, referring to positively charged subunits whose charge is provided dominantly by lysine residues. The two coils, when mixed, form a stable 1:1 K:E dimer. One exemplary E coil sequence is given in Figs. 3B-5B, where the E coil segment constitutes roughly residue numbers 13-53 of the given fusion peptide sequences. The sequence of a K-coil sequence suitable for dimerizing with this E coil is given in the two PCT applications above.

Alternatively, the coiled-coil segment may be a homodimer sequence, referred to herein as an H coil, capable of dimerizing with itself. Exemplary H coil sequences are given in Figs. 3A-5A, where the H coil segment constitutes roughly residue numbers 13-53 of the given fusion-peptide sequences. When mixed, these two segment form a 1:1 H:H homodimer.

To produce a heterodimer modified pilin peptide, fusion proteins containing both E-coil and K-coil N-terminal segments are formed, then mixed to produce the desired dimer. The two different peptides forming the dimer may be modified pilin protein from the same strain, e.g., a PAK/PAK pilin dimer, or from two different strains, e.g., a PAK/K122 dimer. Alternatively, one of the two peptides may be a non-pilin related peptide, for example, a carrier protein that is itself a therapeutic peptide, e.g., peptide anti-bacterial agent, or a carrier protein derivatized with a therapeutic compound that can be cleaved from the carrier, e.g., by an esterase.

In the case of a homodimer, the two modified pilin proteins will in general be same, although homodimers with different strain pilin proteins or with a mixture of a pilin and non-pilin peptide, can be formed in a mixture of same-peptide and different-peptide dimers.

Figs. 2A and 2B show the general construction of expression vectors for recombinant production in an *E. coli* host of modified pilin peptides (fusion peptides) with an N-terminal H coil-truncated pilin fusion peptide, in this case, the PAK peptide (2A), and an N-terminal E coil-truncated PAK pilin fusion peptide 2B). The vectors are constructed according to standard procedures, by inserting a suitable coding sequence into the pRLD vector cut with EcoR1 and HindIII [33, 34]. The polynucleotide sequence given in Figs. 3-5 illustrate exemplary coding sequences for the fusion proteins that are inserted into the vectors.

Figs. 3A and 3B show the nucleotide and polypeptide sequences of the N-terminal H coil-truncated PAK peptide (3A), and the N-terminal E coil-truncated PAK peptide (3B) in the vector constructs in Figs., 2A and 2B, respectively. The nucleotide and polypeptides sequences are identified by SEQ ID NOS: 11 and 12 (H coil peptide), and SEQ ID NOS: 13 and 14 (E coil peptide), respectively. Figs. 4A and 4B show nucleotide and polypeptide sequences for the H-coil and E-coil fusion proteins, respectively, where the sequences are identified by SEQ ID NOS: 15 and 16 (H-coil peptide), and SEQ ID NOS: 17 and 18 (E coil peptide), respectively. Figs. 5A and 5B show nucleotide and polypeptide sequences of the N-terminal H coil-truncated PAO peptide (5A), and the N-terminal E coil-truncated PAO peptide (5B), respectively, where the nucleotide and polypeptides sequences are identified by SEQ ID NOS: 19 and 20 (H coil peptide), and SEQ ID NOS: 21 and 22 (E coil peptide, respectively).

The modified pilin peptide of the invention may be further modified to reduce or eliminate its immunogenicity in humans. This can be done, for example, following the approach disclosed in PCT application WO 98/52976, which is incorporated herein by reference. Briefly, the approach involves the steps of (a) determining at least part of the amino acid sequence of the protein, in this case, modified pilin peptide, (b) identifying in the amino acid sequence one or more potential epitopes for T-cells (T-cell epitopes) of the given species; and (c) modifying the amino acid sequence to eliminate at least one of the T-cell epitopes identified in step (b) thereby to eliminate or reduce the immunogenicity of the protein when exposed to the immune system of the given species.

II. Production and Characterization of Modified Pilin Proteins

A. Preparing the coding sequence and construction of expression vector.

In one general method, modified pilin proteins are prepared by PCR amplification of known and available pilin coding sequences using primers that effect the desired deletion, modification or insertion of a coiled-coil moiety in the amplified coding sequences. The primers also provide suitable

endonuclease cutting sequences at the amplified fragment termini for introduction into selected insertion sites of an expression vector. After amplification and endonuclease treatment, the coding sequence fragment is purified and placed in a suitable expression vector, e.g., an E coli expression vector, under the control od a suitable promoter, for host-cell expression. Example 1 below details construction of the coding sequence and an expression vector for the truncated PAK pilin peptide whose sequence in shown in Fig. 1A.

Example 1

Polymerase chain reaction (PCR). PCR was performed in Stratagene Robocycler 40, thermocycler, using the standard protocol. Each reaction mixture (a total of 100ul) containing the reaction buffer 700mM Tris HCL pH8.8, 200mM $MgCl_2$, 200uM each dNTP, template DNA (1ug), 825ng of each of the primers and 2.5units of Taq polymerase were denatured for 10min at 94°C, followed, by 30 amplification cycles (3min denaturation, at 94°C, 2min annealing at 58°C and 2min extension at 72°C.

DNA sequencing. DNA from the cloned plasmid preparations were sequenced using the dideoxy nucleotide method of Sanger et al., in combination with appropriate oligonucleotides used as primers.

Truncated K122-4 pilin protein gene. Truncated K122 (1-28) pilin gene was engineered by using polymerase chain reaction (PCR). First, previously cloned K122 DNA (22) containing pilin gene was subjected to PCR using the synthetic oligonucleotides primers (with restriction sites added for cloning purposes) flanking the beginning and end of the nucleotide residues corresponding to the amino acids 28 and 150. The resulting PCR product was purified by electrophoresis on an 8% polyacrylamide gel. Fragments of about 380 bp were isolated, and digested with Ecor1 and HindIII enzymes. The digests were purified by Phenol-extraction followed by ethanol precipitation. Fig. 1A shows the polynucleotide sequence, and corresponding amino acid sequence of the truncated pilin protein.

The purified digests were cloned into pRLD expression vector at Ecor1-HindIII sites. The ligated plasmid DNA was transformed into an expression host BL21 strain. Plasmid DNAs were isolated from the Carbenicillin resistant recombinants by the cleared lysate method, and digested with restriction enzymes to check for the correct size inserts. Recombinant DNA containing the correct size inserts were sequenced from both the orientations as described above.

B. Expression of modified pilin protein.

The recombinant protein is expressed in a suitable host under suitable expression conditions, according to well-known methods. For example, for bacterial synthesis, the protein may be obtained

in the periplasmic space of the bacteria, or in secreted form in the host-cell culture medium. Example 2 below illustrates the expression of the above truncated PAK pilin protein in *E. coli*.

Example 2

E. coli cells (BL21) harboring the K122(1-28) plasmid containing K122 truncated pilin gene were grown at 37° C, with shaking in LB medium containing carbenicillin (100μg/ml) to an A50 of 0.5-0.7. Production of recombinant protein was induced by the addition of isopropyl ß-D-thiogalactopyranoside (IPTG) to a final concentration of 1mM. The bacteria were then grown for an additional 8 hours at 37°C. The expressed periplasmic protein was extracted by osmotic shock as follows: The cells were harvested at 4,000g for 10min, at 4°C and re-suspended in TES buffer (10mM Tris-HCl, 5mM EDTA, 20% sucrose, pH 8.0.)in a final volume of 80ml per gram of wet weight. Cells were shaken gently at room temp(150rpm) for 10min. The suspension was then centrifuged and the resulting pellet was re-suspended in 5mM ice-cold $MgSO_4$ (80ml per gram of wet weight). The cell suspension was shaken gently for 30min on ice, and subsequently centrifuged at 8,000g for 15 min at 4°C. The supernatant continuing the periplasmic fraction was then further clarified by passing through a 0.45μm filter and subsequently purified by the column chromatography as described.

C. Protein Purification.

Methods that have been reported for purification of *Pseudomonas* pilin peptide are suitable, although some modification to accommodate the modified sequence may be required. In the case of a fusion pilin peptide having an N-terminal coiled-coil peptide moiety, the fusion protein can be isolated by affinity chromatography, using an immobilized coiled-coil peptide to capture the fusion protein. Fig. 6 illustrates a general scheme for purifying a modified pilin protein formed in accordance with the invention, as detailed in Example 3 below. It will be appreciated that the protein purification scheme is exemplary only.

Example 3

The periplasmic fractions from the transformed bacterial expression host cells were filtered through 0.45μm filter and diluted with an equal volume of 20 mM sodium acetate pH 4.5 buffer and then adsorbed to a carboxymethyl-cellulose column (CM-52 of 30cm x 2cm) which has been previously equilibrated with 10mM sodium acetate pH 4.5 buffer (base buffer) and eluted with a linear gradient of 0-0.8M NaCl in 10mM sodium acetate pH 4.5. Fractions (3ml volume) were collected and the absorbance at A280 nm determined. Fractions containing pilin protein were pooled, freeze dried and dissolved in small amounts of distilled water, and further fractionated on a Sephadex G-75.

As seen in Fig. 7, the molecular weight of the pilin protein (star on the plot) was about 13KD, consistent with the 129 amino acid residue length of the protein (see Fig. 1A).

The isolated protein was fractionated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE gels). Gels were subsequently stained with Coomassie brilliant blue R-250 or transferred to PVDF membranes. Membranes were incubated with K122 IgG antiserum. The secondary antibody used was anti-mouse IgG alkaline-phosphatase. Bound antibody was detected with BCIP substrate. The results (not shown) indicate a substantially pure protein.

D. Ability of fusion protein to compete with native pili for binding to receptor sites.

To confirm that the modified pilin peptide, including dimerized forms of the peptide, are capable of competing with pili for binding to receptor sites, the peptide may be tested in a competitive binding assay with native pili. The pili may be from same-strain or different-strain *Pseudomonas* organisms. Further to this point, the modified pilin can be tested against a number of different-strain pili, to test the cross-specificity the peptide is likely to have as a therapeutic agent. Example 4 below describes an exemplary binding assay of this type.

Example 4

A polystyrene microtitre plate (Costar, Cambridge, MA) was coated with 50 μl/well of asialo-GM_1 (40 μg/ml) in methanol. The solvent was evaporated at room temperature inside a fumehood. Non-specific binding sites were blocked with 200 μl/well of 5% (w/v) BSA in PBS. After incubating at 37°C for 1.5 hours, the wells were washed 3 times with 250 μl of PBS supplemented with 0.05% (w/v) BSA (Buffer A). Aliquots (50 μl) of biotinylated *P. aeruginosa* PAK pili (0.88 mg/ml, diluted 1:1000 in Buffer A) containing various concentrations of K122-4 truncated pilin were added to each well. After a 2 hour incubation at 37°C the wells were washed 5 times with 250 μl of Buffer A, then 50 μl/well of streptavidin-alkaline phosphatase conjugate (Gibco BRL) at 1:3000 dilution with Buffer A was added and incubated for 1 hour at room temperature. Following incubation, the plate was washed 5 times with 250 μl/well of Buffer A. Following washing 80 μl/well of p-nitrophenylphosphate substrate solution (1 mg/ml in 10% diethanolamine, pH 9.8) was then added. Readings at 405nm were recorded and the results were expressed as percent inhibition.

As seen in Fig. 8., the percent inhibition was dose dependent on the concentration of the truncated protein.

III. Treatment Method

The modified pilin peptide composition of the invention is useful in treating existing *Pseudomonas* infection, or as a prophylactic treatment for an individual at risk of *Pseudomonas*

infection, e.g., cystic fibrosis patients, burn patients, and severe neutropenic patients (e.g., cancer patients receiving chemotherapy) and intensive care unit patients receiving respiratory support.

The peptide that is administered in the method may be modified in its N-terminal segment by deletion, substitution, or dimer-forming moieties, as detailed above. Further, the peptide may be modified, e.g., as detailed in WO98/52976 for reduced immunogenicity.

One preferred method of administration is by inhalation, typically in an aerosolized or microparticle form. Methods for preparing and administering peptides by aerosol are well known and suitable for this method. Typically, the amount of peptide administered is between about 0.5 to 25 mg/dose/patient, with the amount of peptide reaching the pulmonary airways depending on the efficiency of the aerosol and administration procedure. The peptide may be administered periodically, e.g., every 6-8 hours, over a period until a satisfactory therapeutic end point is reached.

Alternatively, the peptide may be administered by transmucosal route, e.g., intranasally, or through intravenous (IV), intraperitoneal (IP), intramuscular (IM), or subcutaneous (SubQ) injection. Typically, doses in an amount of 1 mg to 50 mg, administered once a day or over a more frequent dosing schedule, are suitable, although higher doses may be required for IP, IM, or SubQ administration, do to the relatively slow peptide release and uptake at sites if infection. Transdermal administration may also be effective assuming that the peptide can be taken up efficiently by this route. Example 5 below demonstrates therapeutic efficacy when the peptide is administered intraperitoneally.

For prophylactic administration, the peptide may be administered in a single dose, or multiple doses, e.g., every 8 hours, for a period preceding elevated risk of infection, at peptide doses similar to those given above.

In the treatment method, the peptide may be administered in conjunction with anti-bacterial agents, typically non-peptide agents, conventionally used to treat *Pseudomonas* infection.

Example 5

A.BY/SnJ mice were used as they are less resistant to *P. aeruginosa* infection than other mouse strains. Weight:18-20 grams; age:10 weeks. K122-4 truncated pilin (Fig. 1A) was administered intraperitoneally to A.BY/SnJ mice 15 minutes prior to the mice being challenged intraperitoneally with PAK wildtype at $3LD_{50}$. Mice were monitored on a hourly basis between 16 and 48 hours. As seen, percent survival was dose dependent within the range of pilin protein amounts tested.

From the foregoing, it can be seen that (i) an N-terminal modified pilin protein unable to oligomerize (self-assemble) is readily expressed as a secreted processed protein in a recombinant expression system; (ii) the modified protein retains a functional epithelial cell receptor binding domain that mediates binding to GalNAcGal containing glycoconjugates; (iii) the modified protein is

monomeric at protein concentrations of < 600 μg/ml; and (iv), pre-administration of the modified protein confers a dose-dependent protection from challenge with a heterologous *P. aeruginosa* strain in mammals.

Although the invention has been described with respect to specific embodiments, it will be appreciated that the a variety of different pilin peptide N-terminal modifications, and a variety of different *P. aeruginosa* strains may be employed without departing from the invention.